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珙桐中一个与低温相关基因的克隆及其表达研究*

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摘要：低温诱导膜蛋白是由低温诱导表达蛋白基因编码的一类疏水性蛋白，在植物抵御寒冷环境时起着一定的保护作用。从珙桐 cDNA 文库中获得一个未知基因（*DiRCI*），该基因长 539 bp，其中包括 174 bp 的开放阅读框，92 bp 的 5′末端非编码区和 273 bp 的 3′末端非编码区，编码 57 个氨基酸残基蛋白，在氨基酸水平上同源性较高的是车前草的低温诱导膜蛋白（登入号：ACA66247.1），其相似性为 89.5%。半定量 RT-PCR 分析发现，25℃以上，该基因在珙桐各器官中基本上均未表达，但经 8℃低温处理时，在成熟叶片、叶柄和成熟未萌发的种子中均有表达，但是在根中基本没有表达，进一步研究发现该基因在 24 h～48 h 内表达量增多并达到最高值，48 h 后其表达量逐渐减弱直至消失，说明该基因确实与低温诱导相关，从而初步推测该基因为低温诱导膜蛋白基因。该基因的克隆丰富和保存了珙桐基因资源，并为进一步研究冷胁迫的分子机制奠定了基础。

关键词：珙桐；基因；克隆；低温诱导；半定量 RT-PCR
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Cloning and Expression of a Cold-induced Gene (*DiRCI*) from *Davidia involucrata* (Davidiaceae)

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Abstract: A cold-induced plasma membrane protein which plays a protective role in avoiding freezing injury for some plants is one of hydrophobic proteins. A gene, named as *DiRCI*, was isolated from the cDNA library of *Davidia involucrata*, containing an open reading frame of 174 bp flanked by a 92 bp 5′-untranslated sequence and a long 273 bp 3′-non-coding region. Alignment analysis indicated that the deduced amino acid sequence of 57 amino acids was high conserved with the *MpRCI* from *Plantago asiatica*, and the identity was 89.5%. Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis showed that DiRCI wasn′t detected in various organs of *D. involucrata* above 25℃. When the plant grew under 8℃, the gene expressed in leaves, leafstalk and seeds, but not the roots. The expression level gradually increased after 4 h of the cold treatment and reached a maximum during 24—48 h, and then decreased. The results indicated the gene did have a relation with cold stress in plant and was predicted to be one *DiRCI* gene. The present data not only enrich gene resources of *D. involucrata*, but also laid a foundation for the research on molecular

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mechanism of plant acclimation to cold stress.

Key words: *Davidia involucrata* ; Gene; Cloning; Cold-induced; Semi-quantitative RT-PCR

Cold stress is an important factor restricting the geographic distribution and the production of many plants. Acclimation of plants to cold is an adaptive process involving physical, structural and biochemical changes (Wanner and Junttila, 1999; Zhang *et al*., 2005). In general, the cell membrane is the most direct target of low temperature injury in plants, so plants must increase their cryostability of the plasma membrane during cold acclimation process in order to minimize freeze-induced irreversible fusion of membranes (Uemura and Steponkus, 1997). Cold acclimation protein (CAP) is one type of proteins to raise effectively the cryostability of plasma membrane via regulating the synthesis of proteins and the folds of mRNA when plants are stressed by low temperature (Colucci and Inniss, 1996; Yang *et al*., 2003). A great number of genes encoding CAP have been cloned from both monocotyledon and dicotyledon species so far (Sharma *et al*., 2005; Breton *et al*., 2000). Studies on the functions of the genes and their mechanisms in regulating plant acclimation to cold stress using molecular biological techniques will be helpful to breed stress-tolerant plants.

Davidia involucrata Baillon known as the "dove tree" and a "botanic living fossil", is a monotypic genus belonging to the family Davidiaceae. It is a relict from the tropical flora of the Tertiary Period and endemic to China, so it is classified as a first-grade state protection plant (Li, 2003). Although the diverse researches on population genetics (Song and Bao, 2004), taxonomic position (He *et al*., 2004), chemical components (Liu and Ou′Yang, 2006) and tissue culture (Luo, 2006) of *D. involucrata* , no functional genes have been cloned from this endangered species up to date (Qi *et al*., 2009a, b).

To better understand the role of cold-induced genes in enhanced freezing tolerance in *D. involucrata* , a cold-induced gene was cloned from the cDNA library of the plant, then the characteristics of its deduced protein was analyzed, and the expression of this gene was invetigated by semi-quantitative RT-PCR in this study.

1 Materials and methods

1.1 Materials and growth conditions

The fruit of *D. involucrata* and seedlings used in this study were collected from the Wo-Long National Reserve, Dujiangyan City, Sichuan Province. After removing the exocarp, the seeds were air dried and stored at −70℃ before extracting total RNA. Plants were grown in a greenhouse under a light/temperature regime of 16 h/25℃ and 8 h dark/20℃ , and watered daily and irrigated with mineral nutrient solution once every five days.

1.2 Gene cloned from cDNA library of seeds

Based on cDNA library constructed by Qi *et al* . (2009a), a gene sharing high similarity with the *MpRCI* from *Plantago asiatica* (ACA66247.1) aroused our intense interest. This gene, defined as *DiRCI* , was cloned again, sequenced to confirm its sequence, and its expression was analyzed by semi-quantitative RT-PCR.

The PCR primers were designed by Primer Premier 5.0 based on the cDNA sequence. For cloning the ORF of the *DiRCI* , the specific primers are as follows: 1-F: 5′-GAT CCATGG CAT GAG GCA ACA G-3′ (NcoI); 1-R: 5′-CGG TCTAGACAC TTG GTG ATG ACA TAA ACA G-3′ (XbaI). Total RNA was extracted from seeds of *D. involucrata* for synthesizing the cDNA using a reverse transcriptaion kit with Oligo dT as the primers followed by PCR amplification according to the manufacturer′s instructions (Invitrogen, USA). After amplification, PCR products were separated by electrophoresis in 1.5% agarose gel with 1×TAE buffer, stained with ethidium bromide and visualized under UV light. The expected fragments of PCR products were harvested and purified from gel using a DNA harvesting kit (Omega, China), and then ligated into a pDM19-T vector at 16℃ for 1 hours. The recombinant molecules were transformed into *E. coli* complete cells (DH5 α), and then spread on the LB-plate containing 50 $\mu g \cdot ml^{-1}$ ampicillin, 200 $mg \cdot ml^{-1}$ IPTG and 20 $mg \cdot ml^{-1}$ X-gal. Plasmid DNA was isolated and digested by

NcoI and XbaI to verify the insert size. Plasmid DNA was sequenced by Tiangen Corporation (Beijing, China).

1.3 Semi-quantitative RT-PCR

Mature seeds free of the exocarp and seedlings of *D. involucrata* were exposed to 8℃ for 0－64 h under the same light and photoperiodic conditions as described above, then the leaf, leafstalk, root and mature seed were harvested individually at different periods of time, frozen immediately in liquid nitrogen and stored at －80℃ until the isolation of RNA.

The total RNA was extracted from mature leaves, leafstalks, roots and mature seeds for Semi-quantitative RT-PCRs respectively. The RT-PCRs were performed as described previously (Meadus, 2003; Chen *et al*., 2006). The gene-specific primers for PCR analysis of the cold-related gene transcript were: 2-F: 5′-ATC CTC TTG GCC ATC CTT CTG CCT-3′; 2-R: 5′-GTG GGC TTC ACA TCA TCT CCA C-3′. The PCR conditions were 94℃ for 5 min, followed by 30 cycles of amplification (94℃ for 40 s, 58℃ for 30 s, 72℃ for 30 s), then 72℃ for 8 min. The actin gene was amplified using the primers Actin-F (5′-TGT AGG TGA TGA GGC CCA AT-3′) and Actin-R (5′-ATA CCT GTG GTA CGT CCG CT-3′) as a control. PCR products were analyzed on 1.5% (w/v) agarose gels.

1.4 Data analysis

The sequence data were analyzed by Vec Screen (http://www.ncbi.nlm.nih.gov/VecScreen/Vec Screen.html), GenScan software (http://genes.mit.edu/GENSCAN.html) and MEGA4.0 (Borland, America). Homologous analysis of the gene cloned from *D. involucrata* was performed using Blast 2.1 (http://www.ncbi.nlm.nih.gov/blast/) by comparing with the relative gene sequences from other species. Encoding region of the DNA sequence was analyzed using ORF finder software (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) and SignalP 3.0 Server (http://www.cbs.dtu.dk/services/SignalP/). The analysis of hydrophobicity or hydrophilicity of the deduced protein was conducted by ANTHPROT 2000. Multiple Sequence Alignment was performed by software DNAstar Lasergene and DNAMAN 6.0. The secondary structure of the protein was predicted by the PHD algorithm in "PredictProtein" (http://cubic.bioc.columbia.edu/predictprotein/) (Rost, 1996).

2 Results

2.1 Analysis of the cDNA of the *DiRCI* gene

After screening 120 effective ESTs from the cDNA library of *D. involucrata* seed, a gene was selected. Blast analysis showed that the cDNA sequence shares a high similarity (89.5%) with the cold-induced plasma membrane protein gene (*MpRCI*) from *Plantago asiatica* (ACA66247.1), so it was defined as *DiRCI* gene. The length of the *DiRCI* cDNA is 539 bp long, containing a 92 bp 5′-untranslated sequence and a long 273 bp 3′ non-coding region, as indicated by the presence of a poly (A) + tail. An ORF of 174 bp encoding 57 amino acids was found in this gene (Fig. 1).

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1    CGGCCGGGGGCTCCTGTTTGTGTTTGTGTGAAGTCCTCTGATTTGTTTGTTATCTGAATT
61   CAGCGATCTGTGCGAATCCTAACAGAGAAAAA  ATG GCA GAT GAG GCA ACA GCC
1                                      M   A   D   E   A   T   A
114  ACC TGA ATA GAC ATC CTC TTG GCC ATC CTT CTG CCT CCT CTT GGA GTT TTC
8     T   C   I   D   I   L   L   A   I   L   L   P   P   L   G   V   F
165  CTT AAG TTT GGT TGC GAG ATG GAG TTC TGG ATT TGT CTG TTG CTG ACC CTG
25    L   K   F   G   C   E   M   E   F   W   I   C   L   L   L   T   L
216  TTT GGT TAC CTC CCT GGT ATC ATC TAT GCT GTT TAT GTC ATC ACC AAG TGA
42    F   G   Y   L   P   G   I   I   Y   A   V   Y   V   I   T   K   *
267  CCCATCATCTCTTCATAATTAGGTGGAGATGATGTGAAGCCCACAATAAAAGTCTCTATT
327  CATTTCTGCTTATGGAAGAGATGGGTTCTAACGGTTTGATGGACAGTGCAGATTTGGCGA
387  ATTCACATTATTTCATTGTGATTGCGTCTGAGTGTTTTTAGTGATTTCATTTTGTTTGATTT
449  TAATTTGCAGTTTAGTGTAGGTTGGAGTTGGACTGGTGGTTGGTTTGTGATTATGGTGTC
509  GGTTGTGTTGTTTAGTGTCTTTTCAAGCTAG
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Fig. 1　Nucleotide sequence of the *DiRCI* gene from *D. involucrata* and the deduced amino acid sequence from its ORF. "*" indicates the stop codon

2.2 Analysis of the ORF of the *DiRCI* gene and the homologies of its deduced protein

An ORF of the *DiRCI* gene about 180 bp long was easily cloned from the seeds of *D. involucrata* RT-PCR and sequenced. The result indicated that the ORF was absolutely consistent with the sequence from the cDNA library of *D. involucrata* . The deduced protein from the ORF sequence showed that the molecular mass and the isoelectric point are 6.34 kDa and 4.18, respectively. Comparing the DiRCI protein with other reported proteins revealed that they are conserved in molecular weight and amino acids number, but show some differences in the theoretical pI (Table 1 and Fig. 2). In additon, the DiRCI was predicted to show high hydrophobicity possessing a signal anchor peptide in its N-terminal end and have two membrane-spanning domains.

2.3 Expression profiles of *DiRCI* in response to cold

Semi-quantitative RT-PCR analysis revealed that the gene transcripts could be accumulated in response to low-temperature treatment, but the temporal and spatial expression of the gene varied in different organs of *D. involucrata* in cold stress conditions. The gene was able to express in leaves, leafstalks and seeds (Fig. 3: A—C),

Table 1 Comparison the physical and chemical parameters of the protein encoded by *DiRCI* gene and associated proteins from different species

Protein Type	GenBank accession No.	Species	Theoretical pI	Molecular weight/Da
DiRCI	——	*Davidia involucrata*	4.18	6341.7
MpRCI	ACA66247.1	*Plantago asiatica*	4.78	6311.7
stress-induced hydrophobic peptide	XP_002329990	*Populus trichocarpa*	4.32	6243.6
plasma membrance protein3	BAG54793	*Puccinellia tenuiflora*	4.32	6355.7
hydrophobic protein LTI6A	EEF50038	*Ricinus communis*	5.87	6123.5
Os07g0635900	NP_001060390	*Oryzasativa*	4.56	6229.6
hydrophobic protein LTI6B	ACG27760	*Zea mays*	6.04	6392.8
plasma membrane protein 3	BAD34658	*Leymus chinensis*	6.49	5945.3
Protein of unknown function UPF0057	ABD33207.2	*Medicago truncatula*	6.49	5946.3
Protein of unknown function UPF0057	ABD33189	*Medicago truncatula*	4.68	6050.4

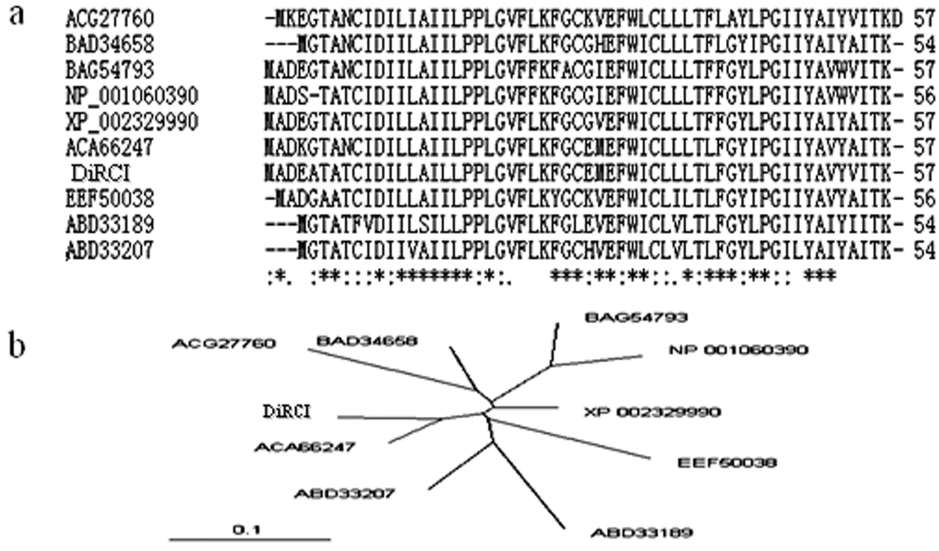

Fig. 2 Multiple alignment (a) and phylogenetic tree (b) of the protein deduced from the *DiRCI* gene from *D. involucrata* and associated proteins. These proteins are indicated with GenBank accession No

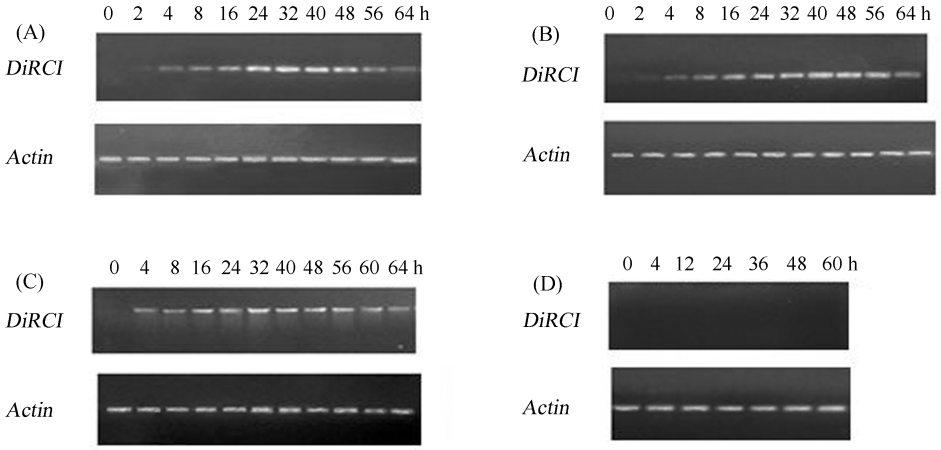

Fig. 3　Low temperature-induced accumulation of the *DiRCI* gene transcripts during different periods of time. RT-PCR analysis was performed with total RNA isolated from leaves (A), leafstalk (B), mature seeds (C) and roots (D) that had been exposed to 8℃ for the indicated time. *Actin* was used as control. The top panel shows the accumulation of the *DiRCI* gene fragment and the bottom panel shows the accumulation of the *Actin* fragment

but not in roots (Fig. 3: D) by 8℃ induction. In leaves, the gene showed an increasing expression after 4 h low-temperature exposure, and reach the maximal lever after 32 h stress, then decreased quickly. Similar to leaves, the expression of the gene in leafstalk were detected after 4 h, and reached the maximum after 40 h of cold stress, and then disappeared gradually. The regularity of changes in mature seeds was similar to those of leaves and leastalks (Fig. 3: C). However, this gene could not expressed in non-induced plant organs above 25℃. The results demonstrated this gene could only expressed transiently in reponse to cold stress in partial organs of *D. involucrata*.

3　Discussion

When induced by a pre-exposure to low but nonfreezing temperatures (Medina *et al*., 2001; Sharma *et al*., 2005), the acclimation of plant to cold is a complex adaptive process. The process requires the systhesis of new proteins (Tseng and Li, 1991), the appearance of new isozymes, alterations in lipid and carbohydrate composition (Schrader *et al*., 2004; Martz *et al*., 2006), the activation of ion channels (Fu *et al*., 2006), the accmulation of compatible osmolytes such as soluble sugars, proline and betaine (Stitt and Hurry, 2002; Uemura *et al*., 2003). Mostly membranes are firstly destroyed by cold stress, especially the plasma membranes (Mahajan and Tuteja, 2005). Efforts to understand the molecular mechanism of cold acclimation have led to the identification of many cold-induced genes in some plants, including *Arabidopsis*, banana (*Musa* sp.), maize, cotton, tomato and so on (Fowler and Thomashow, 2002; Lynch, 1990; Hopkins, 1999), but information about the cold-induced genes encoding integral membrane proteins is very limited.

In this study, we have cloned a cold-induced gene from the cDNA library of *D. involucrata*, *DiRCI*. The gene is predicted to encode a 58 amino acid acidic protein and belongs to the UPF0057 gene family (uncharacterized protein family). The DiRCI protein was predicted to have two membrane-spanning domains with high hydrophobicity. What is more, it shares high similarity with the MpRCI from plantain (Feng *et al*., 2009) and LTI6A from rice (Morsy *et al*., 2005), two member in the UPF0057 gene family, which have been confirmed as integral membrane proteins. However, their theoretical pI

varied in different plants, which may have relationship with the evolutionary development of species and their internal and external environments.

The *DiRCI* gene was induced transiently by low temperature in mature leaves, leafstalks and seeds, but not in roots. The reason may be that the leaves, leafstalks and mature seeds were exposed directly to the outside environment and sensitive to cold, but roots were buried in the soil. The organ specific cold-induced expression of the *DiRCI* gene in *D. involucrata* suggested that the gene mediates an early protective response immediately against sudden stress, before an overall and more permanent response is initiated. Consistent with the report by Feng *et al.* (2009), the results demonstrated that the *DiRCI* gene played a role in resisting to cold and it may be one of low temperature stress defense-related genes in the plant.

The mechanisms of some clod-induced genes have been reported (Gibson *et al.*, 1994; Anderson *et al.*, 1994; Krishna *et al.*, 1995). The transient expression of DiRCI also made a hint it may perform as a regulatory protein to activate other cold relative genes, such as the mitogen-activated protein (MAP) kinase and the calmodulin-related proteins (Mizoguchi *et al.*, 1996; Polisensky and Braam, 1996), but the precise function of the *DiRCI* gene responding to cold stess require further research.

In summary, a *DiRCI* gene, the first gene from *D. Involucrata* , has been cloned and its expression has been investigated. Semi-quantitative RT-PCR results showed that the *DiRCI* gene did have a relation with cold stress. The data will not only enrich and supplement the information about the cold-induced genes but also contribute to the protection for gene resources and the discussion of the genetic polymorphism of this endangered species. However, we get the information that many cold-responsive proteins are also induced by other stresses such as drought, salinity or ABA (Gulick *et al.*, 1994; Capel *et al.*, 1997; Koike *et al.*, 2005). Therefore, it is of interest to determine the expression pattern of the cold-related gene in response to dehydration, salinity and exogenous ABA treatments in future theoretical studies.

References:

Anderson, Li QB, Haskell DW *et al.*, 1994. Structural organization of the spinach endoplasmic reticulum-luninal 70-kilodalton heatshock cognate gene and expression of 70-kilodalton heat shock genes during cold acclimation [J]. *Plant Physiology*, **104**: 1359—1370

Breton G, Danyluk J, Ouellet F *et al.*, 2000. Biotechnological applications of plant freezing associated proteins [J]. *Biotechnology Annual Review*, **6**: 57—99

CapelJ, Jarillo JA, Salinas J *et al.*, 1997. Two homologous low-temperature inducible genes from *Arabidopsis* encode highly hydrophobic proteins [J]. *Plant Physiology*, **115**: 569—576

Chen X (陈昕), Wang BL (王保莉), Qu D (曲东) *et al.*, 2006. Establishment of the semi-quantitative RT-PCR system of sulphur transporter genes in wheat [J]. *Acta Botanica Boreali-Occidentalia Sinica* (西北植物学报), **26** (2): 0309—0313

Colucci MS, Inniss WE, 1996. Ethylene glycol utilization, cold and ethylene glycol shock and acclimation proteins in a Psychrotrophic Bacterium [J]. *Current Microbiology*, **32** (4): 179—182

Feng DR, Liu B, Li WY *et al.*, 2009. Over-expression of a cold-induced plasma membrane protein gene (MpRCI) from plantaim enhances low temperature-resistance in transgenic tobacco [J]. *Environmental and Experimental Botany*, **65**: 395—402

Fowler S, Thomashow M, 2002. *Arabidopsis* transcriptome profiling indicates that multiple regulatory pathways are activated during cold acclimation in addition to the CBF cold response pathway [J]. *The Plant Cell*, **14**: 1675—1690

Fu XY, Chang JF, An LZ *et al.*, 2006. Association of the cold-hardiness of *Chorispora bungeana* with the distribution and accumulation of calcium in the cells and tissues [J]. *Environmental and Experimental Botany*, **65**: 282—293

Gibson S, Arondel V, Iba K *et al.*, 1994. Cloning of a temperature-regulated gene encoding a chloroplast Omega-3 desaturase from *Arabidopsis thaliana* [J]. *Plant Physiology*, **106**: 1615—1621

Gulick PJ, Shen W, An H, 1994. ESI3, a stress-induced gene from *Lophopyrum elongatum* [J]. *Plant Physiology*, **104**: 799—800

He ZC, Li JQ, Wang HC, 2004. Karyomorphology of *Davidia involucrata* and *Camptotheca acuminata* , with special reference to their systematic positions [J]. *Botanical Journal of the Linnean Society*, **144**: 193—198

Hopkins WG, 1999. The physiology of plants under stress [A]. In: Introduction to Plant Physiology, second ed. [M]. New York: Wiley, 451—475

Koike M, Sutoh K, Kawakami A *et al*., 2005. Molecular characterization of a cold-induced plasma membrane protein gene from wheat [J]. *Molecular Genetic Genomics*, **274**: 445—453

Krishna P, Sacco M, Cherutti JF *et al*., 1995. Cold-induced accumulation of hsp90 transcripts in *Brasscia napus* [J]. *Plant Physiology*, **107**: 915—923

Li YX, 2003. Cloning, sequence analysis, and prokaryotic expression of cDNA encoding a putative non-specific lipid-transfer protein from the bracts of Dovetree (*Davidia involucrata* baill) [J]. *Journal of Plant Biology*, **46** (3): 167—172

Liu R (刘荣), Ou′Yang ZA (欧阳明安), 2006. Structural identification of pumiloside, a quinoline alkaloid glycoside, isolated from leaves of *Davidia involucrata* [J]. *Subtropical Plant Science* (亚热带植物科学), **35** (1): 35—38

Luo XJ (罗世家), 2006. Study on tissue culture of *Daviaia involucrata* [J]. *Forestry Science and Technology* (林业科技), **31** (4): 4—6

Lynch DV, 1990. Chilling injury in plants: the relevance of membrane lipids [A]. In: F. Katterman ed., Environmental Injury to Plants [M]. New York: Academic Press, 17—34

Mahajan S, Tuteja N, 2005. Cold, salinity and drought stresses: An overview [J]. *Archives of Biochemistry and Biophysics*, **444**: 139—158

Martz F, Kiviniemi S, Palva ET *et al*., 2006. Contribution of omega-3 fatty acid desaturase and 3-ketoacyl-ACP synthase II (KASII) genes in the modulation of glycerolipid fatty acid composition during cold acclimation in birch leaves [J]. *Journal of Experimental Botany*, **57**: 897—909

Meadus WJ, 2003. A semi-quantitative RT-PCR method to measure the in vivo effect of dietary conjugated linoleic acid on porcine muscle PPAR gene expression [J]. *Biological Procedures Online*, **5** (1): 20—28

Medina J, Catala R, Salinas J, 2001. Developmental and stress regulation of RCI2A and RCI2B, two cold-inducible genes of *Arabidopsis* encoding highly conserved hydrophobic proteins [J]. *Plant Physiology*, **125**: 1655—1666

Mizoguchi T, Irie K, Hirayama T *et al*., 1996. A gene encoding a mitogen activated protein kinase kinase kinase is induced simultaneously with genes for a mitogen-activated protein kinase and an S6 ribosomal protein kinase by touch, cold and water stress in *Arabidopsis thaliana* [J]. *Proceedings of the National Academy of Sciences of the United States of America*, **93**: 765—769

Morsy MR, Almutairi AM, Gibbons J *et al*., 2005. The *OSLti6* genes encoding low-molecular-weight membrane proteins are differentially expressed in rice cultivars with contrasting sensitivity to low temperature [J]. *Gene*, **344**: 171—180

Polisensky DH, Braam J, 1996. Cold shock regulation of the *Arabidopsis* TCH genes and the eVects of modulating intracellular calcium levels [J]. *Plant Physiology*, **111**: 1271—1279

Qi G (齐刚), Su ZX (苏智先), Li JT (李劲涛) *et al*., 2009a. Construction of cDNA library and analysis of the expressed sequenced tags (ESTs) properties of dormant seeds of *Davidia involucrata* [J]. *Scientia Silvae Sinicae* (林业科学), **45** (10): 69—73

Qi G, Li JT, Ruan QP *et al*., 2009b. An optimized, small-scale preparation of high-quality RNA from dry seeds of *Davidia involucrata* [J]. *Phytochemical Analysis*, **20** (2): 139—142

Rost B, 1996. PHD: Predicting one-dimensional protein structure by profile-based neural networks [J]. *Methods in Enzymology*, **266**: 525—539

Schrader J, Moyle R, Bhalerao R *et al*., 2004. Cambial meristem dormancy in trees involves extensive remodelling of the transcriptome [J]. *Plant Journal*, **40**: 173—187

Sharma P, Sharma N, Deswal R, 2005. The molecular biology of the low-temperature response in plants [J]. *Bioessays*, **27**: 1048—1059

Song CW (宋丛文), Bao MZ (包满珠), 2004. Study on genetic diversity of RAPD mark for natural *Davidia involucrata* population [J]. *Scientia Silvae Sinicae* (林业科学), **40** (4): 75—79

Stitt M, Hurry V, 2002. A plant for all seasons: alterations in photosynthetic carbon metabolism during cold acclimation in *Arabidopsis* [J]. *Current Opinion in Chemical Biology*, **5**: 199—206

Tseng MJ, Li PH, 1991. Changes in protein synthesis and translatable messenger RNA populations associated with ABA-induced cold hardiness in potato (*Solanum commersonii*) [J]. *Physiologia Plantarum*, **81**: 349—358

Uemura M, Steponkus PL, 1997. Effect of cold acclimation on membrane lipid composition and freeze-induced membrane destabilization [A]. In: Li PH, Chen THH eds. Plant cold hardiness [M]. New York: Plenum Press, 171—179

Uemura M, Warren G, Steponkus PL, 2003. Freezing sensitivity in the sfr4 mutant of *Arabidopsis* is due to low sugar content and is manifested by loss of osmotic responsiveness [J]. *Plant Physiology*, **131**: 1800—1807

Wanner LA, Junttila O, 1999. Cold-induced freezing tolerance in *Arabidopsis* [J]. *Plant Physiology*, **120**: 391—399

Yang XX, Lin XZ, Li GY *et al*., 2003. Recent advance in psychrophilic lipase and its heterogenous expression [J]. *Shengming De Huaxue (Chemistry of Life)*, **23** (3): 204—206

Zhang CK, Lang P, Dane F *et al*., 2005. Cold acclimation induced genes of trifoliate orange (*Poncirus trifoliata*) [J]. *Plant Cell Report*, **23**: 764—769